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PCSK9 SNP RS11591147 ASSOCIATION STUDY WITH CORONARY ARTERY DISEASE RISK IN IRAN

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ABSTRACT

Pro-protein convertase subtilisin/kexin type 9 (PCSK9) has been implicated in the regulation of the plasma levels of LDL-cholesterol. The SNP rs11591147 variant in PCSK9 gene is associated with low levels of LDL and reduced risk of coronary artery disease (CAD) in various populations. We therefore, investigated the association and protective role of the SNP rs11591147 with CAD in 94 patients and 94 healthy participants as controls. rs11591147 T>G was genotyped in all subjects by TaqMan Probe Real Time PCR, although we could not observe neither positive or insignificant association between SNP genotypes with CAD incidence. Allele frequencies also remained insignificant after performing comparison analyses between cases and controls. LDL-cholesterol and total cholesterol levels were not associated with the genotypes. Our data indicated that CAD or the plasma level of LDL-cholesterol was not associated with the reduction of CAD incidence in Iranian population, even in a population with high frequency of lipid-connected CAD risk factors. Prospective investigations should include more cases to accurately analyze the effect of SNP rs11591147 in protecting patients from CAD.

Key words*: LDL-cholesterol, Atherosclerosis, Variants, Risk factor, Iranian.*

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Introduction

Higher level of blood lipid including circulating low-density lipoprotein-cholesterol (LDL-C) significantly enhances the potential risk for the coronary artery disease (CAD) and myocardial infarction (MI)[(1)]. Increasing concentrations of serum lipids accelerates atherosclerosis and the development of CAD[(2)]. Current experimental data from genetic and epidemiologic studies suggest that inheritance and environmental factors affect the plasma level of lipids. Therefore, to prevent and treat cardiovascular diseases therapeutic researches have been focused on the designation of medicines that lower LDL-C levels[(3, 4)].

Numerous clinical trials have used HMG-CoA reductase inhibitors (statins) as a therapeutic measurement to reduce LDL-C levels and cardiovascular complications, and yet several other drugs are under scientific investigation. In this regard, it is necessary to scrutinize signaling pathways that regulate LDL-C metabolism to discover novel fundamental targets[(5)]. One of these potential targets that influences plasma LDL-C concentration and susceptibility to CAD is pro-protein convertase subtilisin/kexin type 9 (PCSK9)[(6, 7)].

PCSK9 is a circulating protein and by decreasing the half-life of LDL-receptor (LDL-R) controls the plasma levels of LDL-C[(8)]. Mutations in the PCSK9 gene are either associated with the

gene overexpression or deficiency. Upregulation of the gene in mice significantly reduced the amount of LDL-R in liver; however, mice models deficient in PCSK9 demonstrated a correspondingly increased level of LDL-R with active uptake of circulating plasma LDL-C (9-12). Overall, there is a direct relationship between levels of PCSK9 and LDL-C, where PCSK9 overexpression promotes high LDL concentrations and vice versa. PCSK9 is a glycoprotein with 692 amino acids and causative mutations in the gene are known to be highly associated with familial hypercholesterolemia[5,13]. PCSK9 level and thus, LDL-C concentrations vary considerably among individuals and different populations, but it has been suggested that DNA sequence variations of the gene are responsible for this variability and risk of CAD[2,8].

Progressive advances in large-scale genomics' studies have yielded useful approaches such as genome-wide association study (GWAS) to scan for common single nucleotide polymorphisms (SNPs) and their association with a particular phenotype. This technology is capable of whole-genome genotyping to find about 500,000 previously identified SNPs or test for novel genetic loci associated with medical complications[1]. The association of rs11591147 T>G in PCSK9 gene with LDL-C levels and risk of CAD has been shown in several GWAS and replication reports[1,2,5,8,14,16]. Sequence variants with consistent relation to the alterations in blood lipid levels could be used as a valuable prognostic tool to predict the risk for CAD in a subsequent population. Moreover, there is a strong connection between statistically associated SNPs at PCSK9 gene in several populations. Thus, studies of groups from different ethnic origins are required to devise more comprehensive conclusions about interindividual variations and the related effect on CAD. Therefore, this study assessed the association between PCSK9 SNP rs11591147 variants with risk of CAD in Iranian population.

Materials and methods

Study population

The ethics review board of the Shahid Beheshti University of Medical Sciences approved the study; the participants gave written informed consent. 94patients were cases with ages over 40 who underwent coronary angiography and revealed stenosis of ≥ 50% in at least one coronary artery. 94 control group participants were healthy individuals with no previous history of CAD and MI. All subjects submitted a standardized questionnaire regarding conventional cardiovascular risk factors. The collected data included age, sex, cigarette smoking, hypertension (systolic and diastolic blood pressures-SBP and DBP), hypercholesterolemia (HDL-C and LDL-C levels), diabetes (fasting blood sugar-FBS), body mass index (BMI), triglyceride level, height, and weight. Using the High Pure PCR Template Preparation Kit (Roche, Germany), whole genomic DNA was extracted from all blood samples.

Genetic analysis

Spectrophotometry (Nanodrop 1000, Thermo Fisher Scientific, USA) and gel electrophoresis were used to quantify and qualify the extracted DNA. rs11591147 T>G variants were genotyped by TaqMan Probe Real Time PCR (LightCycler 96, Roche, Germany).

Statistical analysis

All procedures were performed by SPSS 21 software (Chicago, USA). The association of the risk factors with CAD was evaluated by testing for the difference between patients and control group by means of a chi-square test for categorical data. Student's t -test was used for continuous data, providing P-values, odds ratios (ORs), and 95% confidence intervals (CIs) to estimate the strength of the association. Comparisons were performed by one-way ANOVA and Kruskal–Wallis test. Additionally, the post hoc test and Mann–Whitney U test were used for comparison across the groups. Multivariate regression test was used to compare the effect of independent variables such as BMI, LDL-C level, gender, and age on SNP genotypes and CAD. Gene counting determined genotype and allele frequencies. Chi-square test was also performed to test whether the genotype and allele frequencies are in accord to the Hardy-Weinberg equilibrium. Values of $P<0.05$ were admitted to indicate statistical significance.

The clinical and biochemical characteristics of the study population are summarized in Table 1. Plasma levels of LDL-C, FBS, and triglyceride were significantly higher among subjects with CAD, but controls revealed an increased level of HDL. The incidence of BMI, hypertension, and mean age remained significantly higher in CAD cases. There was no significant difference between

sex and total cholesterol levels in the groups. No deviation from Hardy-Weinberg equilibrium was detected among the PCSK9 rs11591147 variants in both groups. Table 2 shows the genotype and allele frequencies of the PCSK9 rs11591147 G>T SNP in patients and controls. Among CAD cases all of the patients were homozygous for G allele and none of the cases showed GT or TT genotypes. For the 94 healthy participants included, 91(97%) were with GG genotype while GT heterozygotes constituted 3% of the healthy group (Table 2). Similar to CAD group, we could not observe any healthy participant with the TT genotype.

Variables	Control (n=105)	CAD (n=117)	*P* values
Age (years)	48.28 ± 7.05	58.65 ± 8.89	<0.0001
Sex	0.48 ± 0.51	0.57 ± 0.49	0.2
Body mass index (kg/m2)	25.11 ± 3.08	27.47 ± 6.78	0.002
Systolic Blood Pressure (mm HG)	114.11 ± 10.98	135.81 ± 26.58	<0.0001
Diastolic Blood Pressure (mm HG)	75.32 ± 6.98	83.50 ± 12.75	<0.0001
Triglyceride (mg/dl)	114.00 ± 62.32	155.81 ± 68.72	<0.0001
Total Cholesterol (mg/dl)	171.46 ± 18.16	173.57 ± 32.60	0.5
Fasting Blood Sugar (mg/dl)	85.91 ± 10.79	139.05 ± 62.57	<0.0001
High Density Lipoprotein (mg/dl)	49.52 ± 1.18E+01	39.16 ± 8.10	<0.0001
Low Density Lipoprotein (mg/dl)	87.25 ± 2.47E+01	102.04 ± 24.53	<0.0001

Table 1: Comparison of clinical variables of healthy (control) and CAD patient groups.

Genotype	CAD n (94)	Control n (94)	*p*-value
GG	94 (100%)	91 (97%)	>0.05
GT	0 (0%)	3 (3%)	
TT	0 (0%)	0 (0%)	
G allele	188 (100%)	185 (98.4%)	0.2
T allele	0 (0%)	3 (1.6%)	

Table 2: Genotype and allele frequencies for rs11591147 G>T PCSK9 was tested in patients with CAD and healthy subjects.

Results

Although PCSK9 mutations and underexpression have been discovered in association with reduced level of LDL-C in serum, only few investigations have described and replicated the association of rs11591147 (R46L) genetic variants with potential risk of CAD[2, 15, 17-19]. Benn et al. performed a GWA study and used meta-analysis of previous reports to describe the significant protective role of SNP rs11591147 in ischemic heart disease, MI, and mortality in more than 10,000 participants from white and of Danish descent[20]. The primary purpose of this study was to replicate the association between SNP rs11591147 variants and the risk of CAD in Iranian population. However, this study could not detect a significant association between any variants of the SNP rs11591147 with CAD risk.

Guella et al. 2010 observed the positive association between rs11591147 carriers and lower LDL-C levels, as well as reduced risk for MI among Italians (OR = 0.67; 95% CI = 0.46-0.97; P = 0.036)[2], which is inconsistent with our findings. Another GWAS approach aimed to reveal the association between PCSK9 circulating level with its interindividual variants and plasma LDL in 5,722 subjects in Stockholm. The study confirmed the positive correlation between rs11591147 with decreased plasma levels of PCSK9 (P = 2.20×10-15) and LDL-C (P = 2.05×10-14). There was no relation between the SNP variants with insulin and triglyceride levels[8]. In 1828 whites from the Coronary Artery Risk Development In Young Adults study, Huang and colleagues described the association of rs11591147 variant with LDL-C levels, which was significantly decreased at age 18 in SNP carriers (84.4 mg/dL, $p < 0.001$)[14].

In Cohen's study whites showed similar results to those of Huang report, and rs11591147 carriers revealed about 50% lower CAD occurrence in follow-up period (P = 0.003)[15].

PCSK9 impacts cholesterol levels by inducing LDL receptor degradation, but the mechanism of action of this process is largely unidentified. Studies showed that PCSK9 suppresses LDL receptor to inhibit LDL uptake, but mice depleted for PCSK9 present enhanced LDL clearance[11, 12]. Thus, further work should aim to investigating the relation between PCSK9 variants and assessing the effects by functional assays on LDL metabolism to improve therapeutic applications of CAD complications.

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